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Drug Discovery

Molecular simulation of bioactives from Jimsonweed (*Datura stramonium*) against *Plasmodium falciparum* - glutathione-S-transferase

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To Cite:
Ayodele PF, Adeniyi IA, Bamigbade AT, Omowaye OS, Seweje AJ, Adejoh ME. Molecular simulation of bioactives from Jimsonweed (*Datura stramonium*) against *Plasmodium falciparum* - glutathione-S-transferase. *Drug Discovery* 2024; 18: e8dd1972
doi: https://doi.org/10.54905/dissi.v18i41.e8dd1972

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Peer-Review History
Received: 20 November 2023
Reviewed & Revised: 24/November/2023 to 02/February/2024
Accepted: 06 February 2024
Published: 10 February 2024

Peer-Review Model
External peer-review was done through double-blind method.

Drug Discovery
pISSN 2278–540X; eISSN 2278–5396

ABSTRACT

Introduction: Jimsonweed is a medicinal plant. Malaria is a crucial health problem that is accountable for several deaths yearly across the globe. Understanding the mechanism of *Plasmodium falciparum* and the structure of its receptor where a molecule will bind helps with more approaches to developing new antimalaria drugs. *Plasmodium falciparum*-glutathione-S-transferase (PfGST) plays a role in host cell invasion and evasion of the host immune response. *Materials and Methods:* Gas chromatography-mass spectrometry (GC-MS) of Jimsonweed leaf aqueous extract was carried out. The GC-MS revealed the presence of eighteen compounds. Four of the identified compounds by GC-MS were docked with PfGST. Molecular simulation with PfGST revealed docking scores of -6.8 kcalmol^{-1} (scopolamine), -10.7 kcal/mol^{-1} (withametelin F), -8.0 kcal/mol^{-1} (withametelin H), and -6.5 kcal/mol^{-1} (atropine). *Results:* Withametelin F showed the highest binding tendency with PfGST, the PfGST-withametelin F interaction was viewed in Biovia Discovery Studio. Amino acid residues involved in the intermolecular interaction include Lys 207 and Tyr 30. *Conclusion:* Our findings revealed the stability of withametelin F on PfGST. This reveals that, leaves of Jimsonweed are promising sources for the search for new drugs against *Plasmodium falciparum*.

Keywords: Jimsonweed; Molecular simulation; Glutathione-S-transferase; *Plasmodium falciparum*; PfGST

1. INTRODUCTION

Many plants have long been in use as traditional medicine (Yasir *et al*., 2023), it is crucial to have good understanding of these plants and their phytochemical contents

before using them (Thakur *et al*., 2023; Uddin *et al*., 2023). Phytochemicals play pivotal roles in sustaining several metabolic functions. These functions have addressed a plethora of chronic ailments, including diabetes, blood pressure irregularities, central nervous system disorders, and cancer, and in the treatment of several types of microbial diseases such as bacterial, parasitic, fungal, and viral diseases (Tom-Otu *et al*., 2022). Bioactives from plants act as nature's sentinels, guarding plants against microbial assailants, irritating pests, herbivores, and the capricious whims of changing environmental conditions. Beyond these significances, these bioactives possess remarkable disease-preventive and curative properties (Singh *et al*., 2019), though their effects can range from highly beneficial to potentially detrimental.

Jimsonweed belongs to the *Solanaceae* family and has a wide array of therapeutic applications, such as the treatment of certain types of nerve agent and pesticide poisonings, bladder spasms, peptic ulcers, diverticulosis, colic, irritable bowel syndrome, and inflammation of the pancreas. However, several findings have also revealed that some parts of the plant are poisonous (Thakur *et al*., 2023), especially when consumed at higher concentrations. Molecular simulation plays a role in discovering chemical compounds from natural origin (Baig *et al*., 2020). It has been an undoubtedly useful tool in drug discovery and the study of biological systems (Ayodele *et al*., 2023); it plays a crucial role by allowing researchers to understand how potential drug candidates interact with their target proteins at the molecular level. This helps in designing more effective and specific drugs (Khan *et al*., 2020). However, understanding the etiology of disease and the structure of its receptor where a ligand will bind helps with more approaches to developing new drugs.

Thus, it has been the goal of medicinal chemists to predict biological activity in untested compounds and define the structural requirements for a good fit between a drug molecule and a specific receptor by an *in-silico* screening of drug–receptor candidate interactions.

Malaria is a common and life-threatening disease in the tropics, sub-tropic, and outside sub-Saharan African regions of the globe (Afolabi *et al*., 2021; WHO, 2020). It is caused by the infection of *Plasmodium falciparum*, a protozoan, among other species that are transmitted by the female Anopheles mosquitoes (Afolabi and Oyewole, 2020; Omagha *et al*., 2023). Malaria is a major public health problem that poses serious socioeconomic challenges (Abamecha *et al*., 2020; WHO, 2020).

Glutathione-S-transferase (GST: EC 2.5.1.18) (also known as ligandin), is a family of prokaryotic and eukaryotic phase II metabolic isozymes. The enzymes are best known for their ability to catalyze the conjugation of reduced glutathione (GSH) with xenobiotic substrates for detoxification (Nimse and Pal, 2015).

Plasmodium falciparum contains GST encoded by the parasite's chromosome 14. The role of GST from *P. falciparum* (PfGST) in the development of drug resistance in malarial parasites has been postulated and is controversially discussed (Harwaldt *et al*., 2002). The parasite harbors only one GST and inhibition of PfGST is expected to disturb GSH-dependent conjugation processes, enhance levels of cytotoxic peroxides, and increase the concentration of toxic ferriprotoporphyrin IX (FP). The PfGST is a most promising drug target (Deponte and Becker, 2005). The huge challenge in malaria treatment, in which *Plasmodium falciparum* has developed resistance to available treatments, makes the identification of new target proteins and bioactive(s) that can inhibit the synthesis of the proteins a fire-approach mission for the development of novel anti-malaria drugs (Ruiz-Carrillo *et al*., 2018), from less explored natural products.

2. MATERIALS AND METHODS

Leaves Collection

Leaves of Jimsonweed were harvested in a residential environment at Oko-Irese, Kwara State, Nigeria. Botanical identification and authentication of the plant (TAU-108/3) were conducted by a plant scientist in the Department of Biological Sciences, Thomas Adewumi University, Oko, Kwara State, Nigeria. The leaves were rinsed, sorted, and air-dried until crispy to the touch, after which they were pulverized into a fine powder using a sterile electrical blender (Euro premium: 1500-1799 W).

Preparation of Leaves Extract

Five grams (5 g) of the pulverized sample was cold macerated in 50 ml distilled water (1: 10 w/v) for a period of 48 hours with continuous rocking on a control orbital shaker with model KS 260. The aqueous extract was filtered using a Whatmann Number 1 filter paper of 100 (195 mm by 195 mm) pore size to obtain a clear solution. The filtrate was concentrated using a rotary evaporator and placed in a water bath at 40^0C for further evaporation of the solvent. The prepared sample was taken for qualitative and quantitative phytochemical analysis using gas chromatography–mass spectrometry (GC-MS).

GC-MS of the Leaves Aqueous Extract

The bioactive compositions of Jimsonweed leaf aqueous extract were assessed using a Trace GC 1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG-5MS (30 m × 0.25 mm × 0.25 µm film thickness). The column oven temperature was at first held at 60^0C/ 2 min, then 30^0C/min, to 120^0C/3 min, to 30^0C/min, to 150^0C/2 min, to 30^0C/min, then to 250^0C/5 min. The injector and MS transfer line temperatures were kept at 230^0C and 280^0C, respectively. Helium (He) was used as a carrier gas at a flow rate of 1.5 ml per minute with an injection volume of 1µL. The total GC - MS run time was 10.459 minutes. The software used for the analysis was GC-MS-solution software for simultaneous analysis. The bioactives present in the sample were identified using the database of NIST and Wiley Library. The spectrum obtained was compared with the database in the library to obtain qualitative data. The area percentage of the bioactive peaks was used as an indication of the abundance of the bioactives. As more area percentage of the peak indicates more abundance.

Preparation of Protein (PfGST)

Plasmodium falciparum-GST (PfGST) (protein data bank ID: 10KT) was retrieved from https://www.rcsb.org/structure/10KT. The significance of the protein in intra-erythrocytic stages of the *Plasmodium* parasite necessitated the choice of the protein. The protein (PfGST) was prepared using Biovia Discovery Studio Visualizer version 16. Water particles and heteroatoms were deleted from the protein. Polar hydrogen was added and PfGST was saved as PfGST.pdb, in a protein data file.

Preparation of Ligand Structures of *D. stramonium* Leaves

The 3D structures of bioactives derived from *D. stramonium* leaves were retrieved from the PubChem database (www.pubchem.ncbi.nlm.nih.gov) in a structure data format (SDF). The SDF structures of these bioactives were converted to mol-2 chemical format using Open Babel. Polar hydrogen charges of the Gasteiger type were assigned to atoms in the chemical structure, and non-polar molecules of the hydrogen atoms were merged with carbons. The internal degrees of freedom and torsions were set to zero. Structures of the bioactives were then converted to the dockable PDBQT format using AutoDock tools.

Molecular Docking Simulations of Compounds from *D. stramonium* Leaves against PfGST

The *D. stramonium* leaves ligand structures were imported into AutoDock Vina in PyRx 0.8 (Trott and Olson, 2010). Their energy levels were minimized by applying a universal force field (UFF) as the energy minimization parameter and conjugate gradient descent as the optimization algorithm, using the incorporated Open Babel. Scopolamine (PubChem ID: 5184), withametelin F (PubChem ID: 14283156), withametelin H (PubChem ID: 101131180), and atropine (PubChem ID: 174174) were then screened against the active site of PfGST (protein data bank ID: 10KT). The active site of the PfGST was defined by the grid boxes in a vina search space X: 48.8294, Y: 43.7984, and Z: 9.0984; dimensions X: 119.495, Y: 63.5237 and Z: 24.2681. The molecular docking simulations were then analyzed, keeping all other parameters as default. All the ligands with their binding scores were identified (Table 2). Model 1 of the withametelin F interaction outcomes was selected, and its molecular interaction with PfGST was viewed with Discovery Studio Visualizer version 16.

3. RESULTS

Table 1 and Figure 1 show the GC-MS analysis of Jimsonweed leaf. The GC-MS revealed the presence of eighteen (18) bioactive compounds. Table 2 shows the binding affinities of four selected structure-based bioactive compounds (scopolamine, withametelin F, withametelin H, and atropine) from Jimsonweed leaf for PfGST. Molecular simulation of these bioactives with PfGST revealed the docking scores of -6.8 kcalmol^{-1} (scopolamine), -10.7 kcal/mol^{-1} (withametelin F), -8.0 kcal/mol^{-1} (withametelin H), and -6.5 kcal/mol^{-1} (atropine).

Table 1 Qualitative and quantitative phytochemical constituents of Jimsonweed leaf aqueous extract by GC-MS

Peak	RT	Area%	Compound
1	1.396	10.05	Propanal
2	1.470	2.22	Scopolamine
3	1.642	7.49	3-methylbutanamide
4	3.639	10.58	2-Pentanone

5	6.723	2.24	8-methylheptadecane
6	8.182	3.03	n-Hexadecanoic acid
7	8.537	3.35	8,11-Octadecadienoic acid
8	8.600	2.63	Withametelin F
9	8.663	18.14	9,12-Octadecadienoic acid
10	8.708	6.07	Octadecanoic acid
11	8.966	7.40	16-Hexadecanoyl hydrazide
12	9.052	2.29	Atropine
13	9.532	7.68	Stearic anhydride
14	9.572	3.45	1,8,11-Heptadecatriene
15	9.641	2.20	Withametelin H
16	9.704	5.96	Docosanoic acid
17	10.373	2.63	9,12-Octadecadienoic acid
18	10.459	2.61	9-Octadecanoic acid

Table 2 Binding scores of selected ligands from Jimsonweed, screened against PfGST

Ligand/Models	Binding Affinity	rmsd/ub	rmsd/lb
1okt_5184_uff_E=1801.54	-6.8	0	0
1okt_5184_uff_E=1801.54	-6.7	6.7	2.548
1okt_5184_uff_E=1801.54	-6.6	2.005	1.306
1okt_5184_uff_E=1801.54	-6.6	6.653	2.202
1okt_5184_uff_E=1801.54	-6.4	6.552	2.481
1okt_5184_uff_E=1801.54	-6.4	1.829	1.367
1okt_5184_uff_E=1801.54	-6.3	3.71	2.202
1okt_5184_uff_E=1801.54	-6.3	1.821	1.451
1okt_5184_uff_E=1801.54	-6.3	6.599	2.505
1okt_14283156_uff_E=2304.85	-10.7	0	0
1okt_14283156_uff_E=2304.85	-10.3	2.528	1.747
1okt_14283156_uff_E=2304.85	-10	3.899	2.515
1okt_14283156_uff_E=2304.85	-9.6	9.464	3.618
1okt_14283156_uff_E=2304.85	-9.4	2.498	1.863
1okt_14283156_uff_E=2304.85	-9.4	3.178	2.277
1okt_14283156_uff_E=2304.85	-9.1	4.452	3.289
1okt_14283156_uff_E=2304.85	-8.6	26.13	23.004
1okt_14283156_uff_E=2304.85	-8.6	5.639	4.104
1okt_101131180_uff_E=768.21	-8.0	0	0
1okt_101131180_uff_E=768.21	-7.5	2.718	1.766
1okt_101131180_uff_E=768.21	-7.4	10.527	2.509
1okt_101131180_uff_E=768.21	-7.4	3.762	2.197
1okt_101131180_uff_E=768.21	-7.2	2.055	1.669
1okt_101131180_uff_E=768.21	-7.2	3.496	2.32
1okt_101131180_uff_E=768.21	-7.1	3.643	1.956
1okt_101131180_uff_E=768.21	-7.1	8.852	2.233
1okt_101131180_uff_E=768.21	-6.9	10.042	2.271
10kt_174174_uff_E=455.80	-6.5	0	0
10kt_174174_uff_E=455.80	-6.2	27.603	25.95
10kt_174174_uff_E=455.80	-6.1	18.107	15.51
10kt_174174_uff_E=455.80	-6.1	2.092	1.05
10kt_174174_uff_E=455.80	-5.9	7.077	4.316
10kt_174174_uff_E=455.80	-5.8	22.671	20.087
10kt_174174_uff_E=455.80	-5.8	28.298	26.635

10kt_174174_uff_E=455.80	-5.7	31.879	30.574
10kt_174174_uff_E=455.80	-5.7	6.281	4.307

*The lower the binding score, the higher the binding affinity

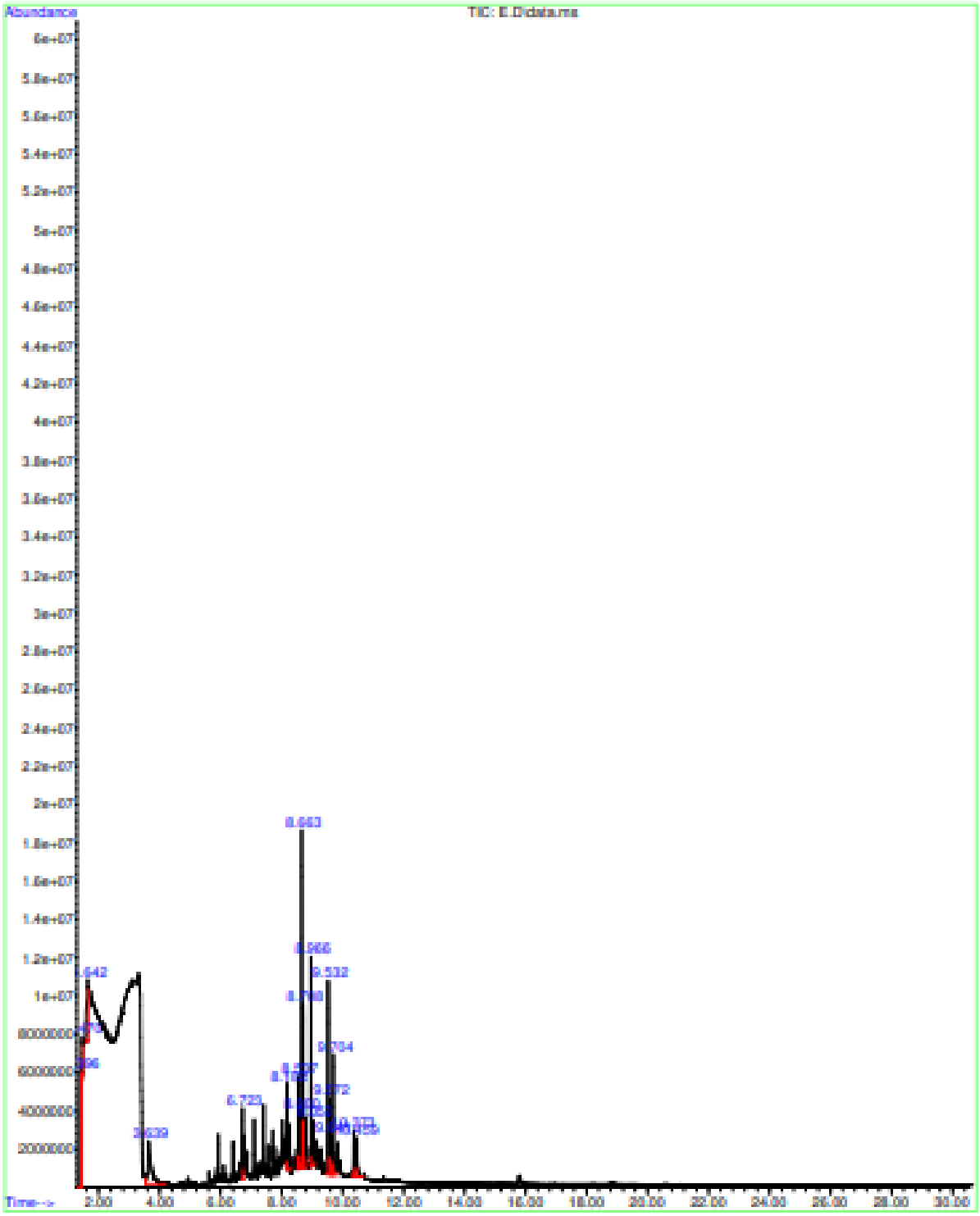
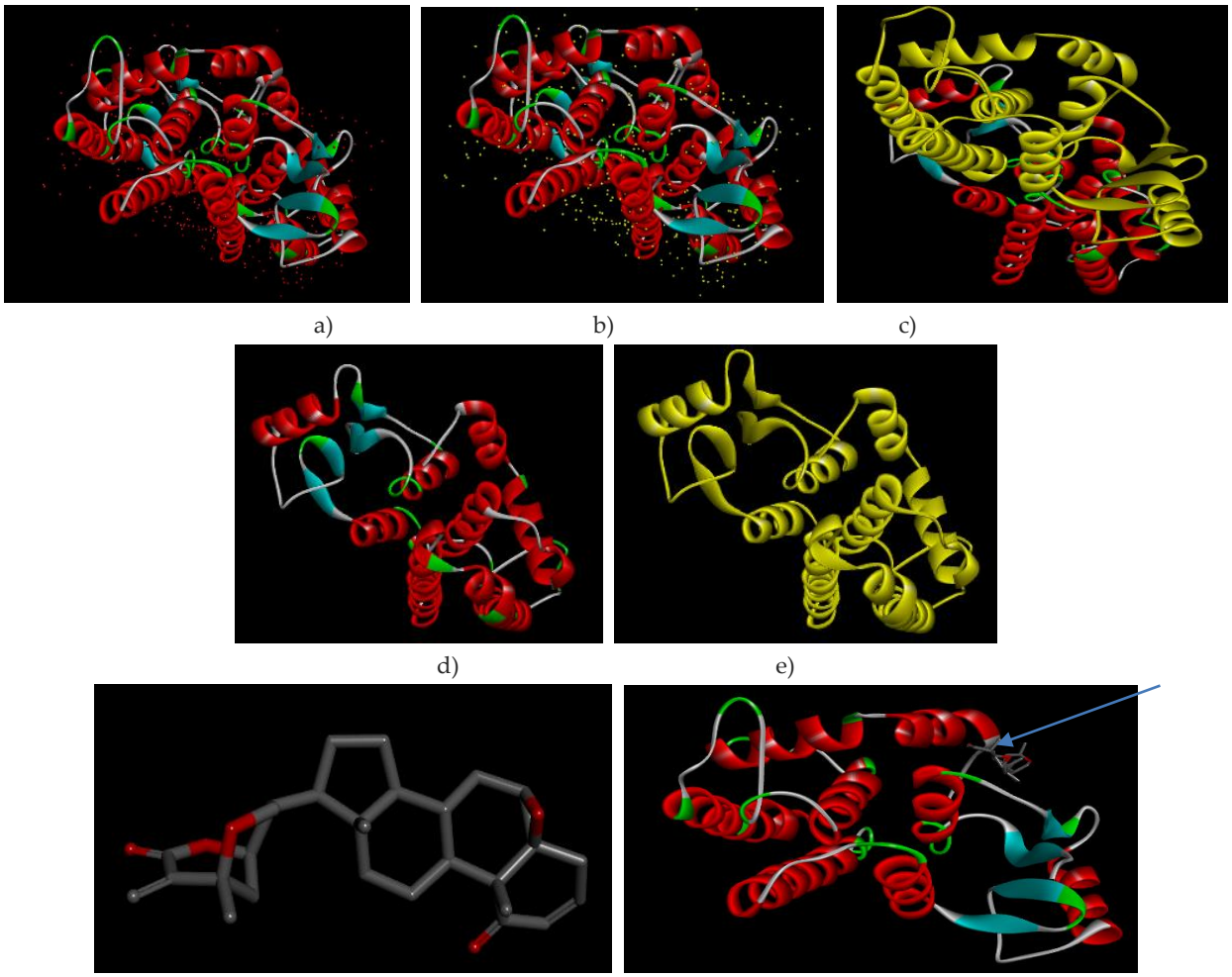


Figure 1 Chromatogram of Jimsonweed leaves aqueous extract by GC-MS



Figures 2 a: PfGST in its original state, b: PfGST + water particles + heteroatoms, c: Dimeic PfGST after water particles and heteroatoms have been removed, d: Chain A of PfGST, e: Chain B of PfGST, f: Withametelin F (PubChem ID: 14283156), g: PfGST-withametelin F interaction

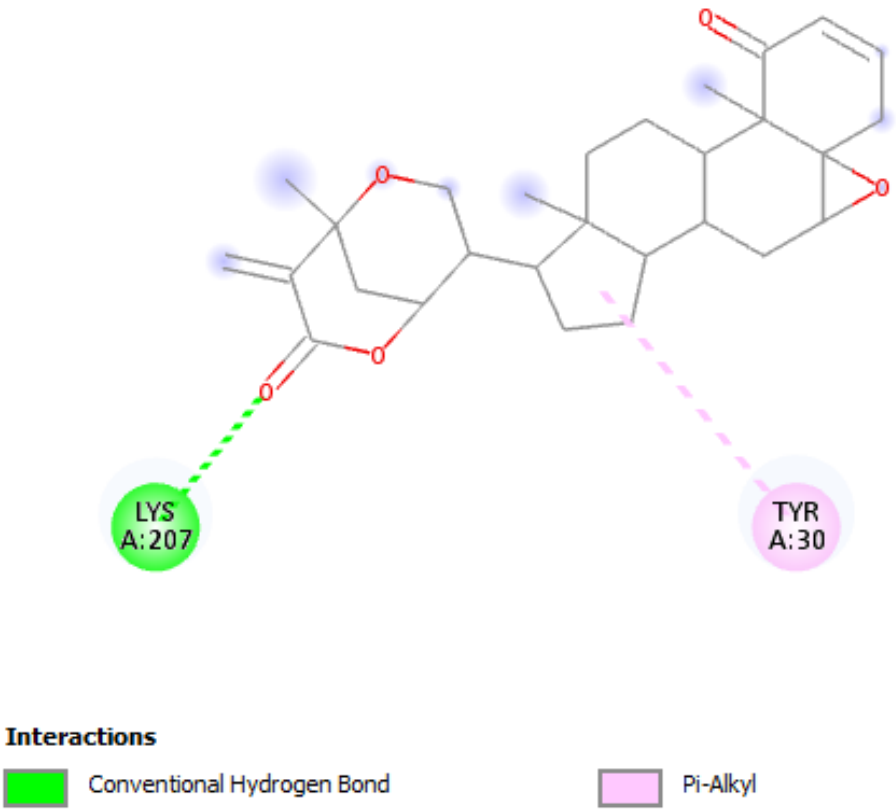


Figure 3 a) 2-D structure of PfGST-withametelin F complex

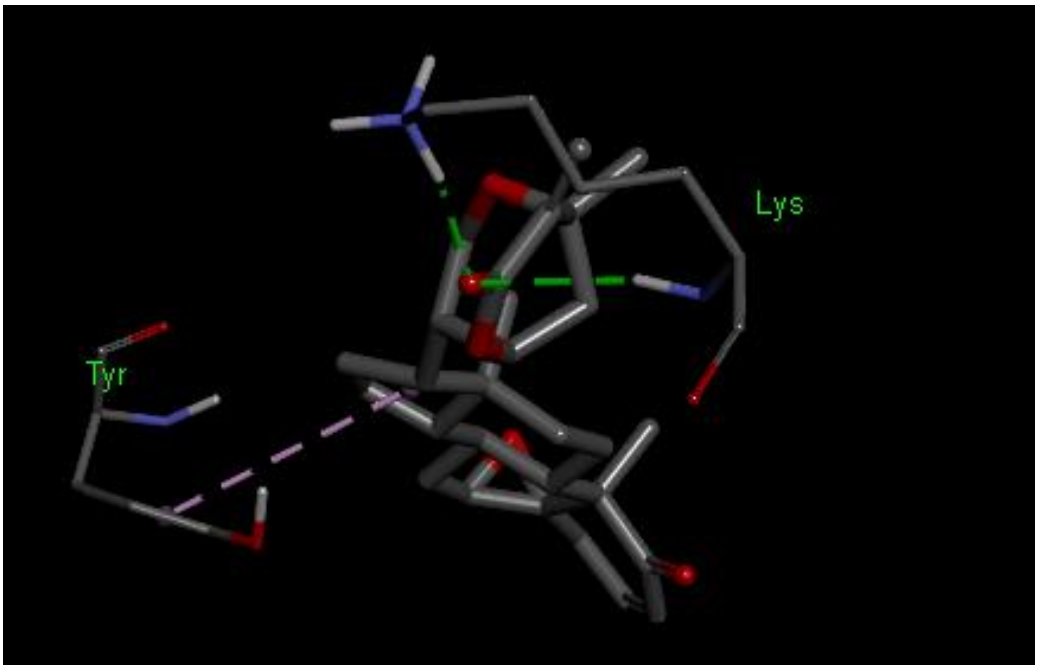


Figure 3 b) 3-D structure of PfGST withametelin F complex

Withametelin F showed the highest binding tendency with PfGST, having the lowest binding score with the PfGST. Figure 2 shows the dimeric PfGST (a: PfGST in its original state, b: PfGST + water particles + heteroatoms c: dimeric PfGST after water particles and heteroatoms have been deleted from the chain, d: Chain A of PfGST, e: Chain B of PfGST, f: structure of withametelin F, g: PfGST-withametelin F interaction). Figure 3a shows the 2-dimensional structure of the PfGST-withametelin F complex, and 3b shows the 3-dimensional structure of the PfGST-withametelin F complex. Amino acid residues involved in the intermolecular interaction include Lys 207 and Tyr 30.

4. DISCUSSION

The medicinal relevance of Jimsonweed cannot be overemphasized. It has wide records of usage in ethnomedicine (Thakur *et al*., 2023; Choudhary *et al*., 2021). The result from the GC-MS analysis revealed the presence of hexadecanoic acid, and 9-octadecenoic acid, including scopolamine and atropine. This agreed with the report of Thakur *et al*. (2023). The bioactive compounds in a particular plant vary within species, age, time of harvest, soil topography, processing activities, extraction solvent, climate, or weather (Oseni *et al*., 2011), this might necessitate the presence of other bioactive compounds found from the plant used in this study. Understanding the mechanism of *Plasmodium falciparum* and the structure of its receptor where a drug-like candidate will bind helps with more approaches to the development of new antimalaria drugs. This will provide a mechanistic approach to tackling malaria and proffer a promising solution to the challenge of drug resistance. Malaria ranks high among the leading causes of mortality in the world, and early diagnosis and treatment may prevent unwanted complications (Afolabi *et al*., 2021).

It is prevalent in Africa and some parts of Asia, and it occurs as an imported disease in developed countries (WHO, 2020). Malaria is caused by parasites of the genus *Plasmodium*, which are transmitted to humans through a bite from an infected female Anopheles mosquito. A severe form of malaria is mainly caused by *Plasmodium falciparum* (Abamecha *et al*., 2020; WHO, 2020). Quinine was the first widely used drug in the treatment of severe malaria; however, due to drug resistance by the parasite, combination therapy is increasingly being employed, and artemisinin derivatives are now recommended for quinine-resistant cases (Jasminka *et al*., 2019; Andrej *et al*., 2003). Although certain malaria vaccines have been recommended for implementation as a childhood vaccine, it remains likely that existing and potentially effective drug candidates will still have a significant place in the management of severe malaria for the foreseeable future (Matthew, 2018). The need to combine antimalaria drugs with chemo modulatory agent (s) has been postulated (Na *et al*., 2007; Hiller *et al*., 2006; Harwaldt *et al*., 2002).

Plasmodium falciparum glutathione-S-transferase is the only GST present in this parasite and is similar to the human mu homolog. According to the literature, PfGST activity is increased in chloroquine-resistant strains, and the enzyme has been revealed to act as a ligandin for the parasitotoxic haemin. The GST activity is influenced by natural plant products. Flavonoids and several naturally occurring plant products have been shown to inhibit PfGST with different IC_{50} (Mangoyi *et al*., 2010). X-ray crystallographic studies have indicated that the GST polypeptide is organized into two binding domains: a GSH binding domain (domain I) located at the N-terminus and a xenobiotic substrate binding domain (domain II) located at the C-terminus.

A combination of techniques including spectroscopy, kinetic study, mutagenesis, and X-ray crystallography research on native and mutant enzymes has revealed that tyrosine (Tyr 6) of domain I and Tyr 115 of domain II of GST participate directly in catalysis (Johnson *et al*., 1993; Liu *et al*., 1992). Although PfGST has no similarity to any of these, it is tilted towards the mu class. Since Tyr 30 of the PfGST is involved as potential binding residue in this study, it is the catalytic residue. It is possible to suggest this inhibitor may likely act as a non-competitive inhibitor (Hiller *et al*., 2006), of course, this is possible since the GST is active as a dimer. Withametelin is a subclass of withanolides and is a natural medicinal agent whose safety and therapeutic profiles make it valuable to humankind. The inhibition potential of withametelin F on PfGST in malaria parasites can thus be associated with its pharmacological properties as reported by Baig *et al*. (2020).

5. CONCLUSION

Relative to the binding of physiological ligands and the potential accessibility for protein inhibitors, the molecular interaction of PfGST with withametelin F from Jimsonweed was studied, and it indeed provides insight into the anti-*Plasmodium falciparum* potency. To the best knowledge, as of the time of collecting these data, no work has been done on the PfGST inhibition potency of withametelin F from Jimsonweed. This calls for further investigation on how a new drug or a new combination therapy other than the existing antimalaria drugs which have developed resistance to the malaria parasite, could be designed from withametelin F.

Acknowledgment

We thank the participants who all contributed samples to the study.

Authors Contributions

Peter Folorunsho Ayodele: Conceptualization, methodology, performing the experiment, data analysis, and drafting the manuscript.
Isaac Abiodun Adeniyi: Conceptual framework drawing, study design, and data collection.
Adekunle Toyin Bamigbade: Drafting the manuscript.
Olaniyi Stephen Omowaye, Maji Emmanuel Adejoh: Review and editing.

Ethical Approval

Leaves of Jimsonweed were harvested in a residential environment at Oko-Irese, Kwara State, Nigeria. The ethical guidelines for plants and plant materials were followed in the study for sample collection and identification (Ethical approval code: TAU-108/3).

Informed Consent

Not applicable.

Conflicts of interests

The authors declare that there are no conflicts of interests.

Funding

The study has not received any external funding.

Data and materials availability

All data associated with this study will be available based on the reasonable request of the corresponding author.

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